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Amygdala–Hippocampal Connectivity Is Associated With Endogenous Levels of Oxytocin and Can Be Altered by Exogenously Administered Oxytocin in Adults With Autism

Kaat Alaerts, Sylvie Bernaerts, Bart Vanaudenaerde, Nicky Daniels, and Nicole Wenderoth

ABSTRACT

BACKGROUND: Oxytocin (OT) plays a pivotal role in interpersonal bonding, affiliation, and trust, and its intranasal administration is increasingly considered as a potential treatment for autism spectrum disorder.

METHODS: We explored whether variations in endogenous salivary OT concentration are related to interindividual differences in core autism symptoms and expressions of attachment in 38 male adults with autism spectrum disorder. Further, resting-state functional magnetic resonance imaging was adopted to specifically explore whether interindividual differences are reflected in the intrinsic network organization of key regions of the central oxytocinergic system.

RESULTS: Positive correlations were identified between peripheral OT and expressions of secure attachment (the State Adult Attachment Measure and the Inventory of Peer Attachment), but no significant relationships were identified with scales assessing core autism symptom domains (the Social Responsiveness Scale and the Repetitive Behavior Scale). At the neural level, higher levels of endogenous OT were associated with lower degrees of interregional functional coupling between the amygdala and hippocampal regions. Interestingly, a single dose of exogenously administered OT induced a further reduction in amygdala–hippocampal connectivity, indicating that a higher availability of OT can alter the degree of amygdala–hippocampal connectivity.

CONCLUSIONS: The identified associations between the oxytocinergic system, expressions of secure attachment, and amygdala–hippocampal pathways are anticipated to be of relevance for understanding the role of OT in modulating appropriate neural and physiological responses to stress and restoring homeostasis.

Keywords: Amygdala, Attachment, Autism spectrum disorders, Hippocampus, Oxytocin, Resting-state fMRI

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The neuropeptide oxytocin (OT) is produced by the paraventricular and supraoptic nuclei of the hypothalamus and is known to play a pivotal role in a variety of complex social behaviors, including interpersonal bonding, trust, and affiliative and cooperative behavior [for reviews see (1–3)]. In the past decade, intranasal administration of OT has gained increasing interest as a possible treatment for targeting the socio-communicative difficulties that are characteristic of autism spectrum disorder (ASD).

Initial single-dose administration studies have consistently demonstrated behavioral improvements on various social tasks in patients with ASD (4–8). Also, several multiple-dose administration studies showed improvements in the social domain after 5 or 6 weeks of continual OT administration in adults (9,10) or in 4- or 5-week trials in children with ASD (11), even though other multiple-dose studies have failed to replicate these positive outcomes after 4-day (12) or 8-week trials (13) in children and adolescents with ASD. Interestingly, in a 2017 study by Parker *et al.* (14) examining the effect of a course of 4 weeks of continual OT treatment in children with ASD it was shown that pretreatment blood OT concentrations predicted treatment response, indicating that individuals with pretreatment OT signaling deficits may benefit the most from OT treatment (14). To date, controversy exists with respect to the association between ASD diagnosis and endogenous OT levels, with initial studies demonstrating lower OT levels in children with ASD compared with control subjects (15–17), while other studies showed no differential effect of diagnosis (18,19) or even higher OT levels in adults with ASD (20). Beyond diagnosis, however, more consistent associations have been demonstrated between endogenous levels of OT in plasma and maternal or paternal bonding behaviors, infant social engagement, attachment-related thoughts, and reduced psychological distress (21–24).

While evidence with respect to associations of endogenous OT levels with behavioral manifestations of interpersonal bonding, attachment, and trust is increasingly emerging, research investigating whether and how interindividual

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variations in endogenous OT are related to variations in the central oxytocinergic brain system is relatively sparse. Mielke *et al.* (25) recently explored associations between plasma OT and brain morphology of the hypothalamus (where OT is produced) and other brain regions of the central oxytocinergic system (amygdala, hippocampus, nucleus caudatus, and nucleus accumbens) and showed that lower levels of OT were associated with larger gray matter volume of the amygdala and hypothalamus in women with early-life maltreatment (25). Similarly, Andari *et al.* (26) also identified an association between lower OT levels and higher gray matter volume in the right amygdala and the right hippocampus. While these structural neuroimaging studies provided initial evidence of a link between endogenous OT levels and structural variations in the central OT system (the amygdala in particular), it remains unclear whether and how endogenous OT levels are related to functional variations of the central OT circuitry.

To fill this gap, the current study explored whether endogenous (salivary) OT levels in male adults with ASD are related to variations in resting-state functional connectivity between key regions of the central oxytocinergic system (amygdala, hippocampus, nucleus caudatus, nucleus accumbens, and hypothalamus). Resting-state functional magnetic resonance imaging (fMRI) data (as opposed to task-evoked fMRI) were acquired because resting-state functional connectivity is postulated to reflect more intrinsic (trait-like) individual differences in circuitry integrity, whereas task-evoked changes are anticipated to reflect more transient (state-like) changes in brain network characteristics in response to specific task demands. Importantly, for the interregional connections that intrinsically showed a significant association with endogenous levels of OT (at baseline), we next specifically explored whether a single dose of exogenously administered OT would be able to yield an alteration in the intrinsic pattern of interregional functional coupling (postadministration).

Aside from these neurophysiological characterizations, we also assessed associations between endogenous OT levels and the degree of core autism symptoms (social responsiveness and repetitive behaviors). Furthermore, and considering previous evidence in neurotypicals of an association between endogenous OT levels and expressions of interpersonal bonding and attachment (23), we also explored whether similar associations between endogenous OT and interindividual differences in self-expressed state and trait attachment are evident in patients with ASD.

METHODS AND MATERIALS

General Study Design and Participants

The principal aim of the current study was to explore associations between endogenous OT levels and neural (resting-state fMRI functional connectivity) and behavioral measures (interindividual variations in core autism symptoms and attachment) in patients with ASD. For the interregional connections that intrinsically showed a significant association with endogenous levels of OT (at baseline), we specifically explored whether a single dose of exogenously administered OT would be able to yield an alteration in the intrinsic pattern of interregional functional coupling.

Forty participants with ASD were recruited from the Autism Expertise Centre at the Leuven University Hospital to participate in this double-blind, randomized, placebo-controlled trial with parallel design (oxytocin group, n = 22; placebo group, n = 18) (Supplemental Figure S1). As visualized in the Consolidated Standards of Reporting Trials diagram, data of 1 participant from the oxytocin group and 1 participant from the placebo group were not included in the final MRI analyses because of insufficient data or low data quality (excessive in-scanner head motion: 98.5% of images with framewise displacement $>$0.5 mm). Written informed consent was obtained from all participants before the study. Consent forms and study design were approved by the Universitair Ziekenhuis/Katholieke Universiteit Leuven Ethics Committee for Biomedical Research (S56327) in accordance to The Code of Ethics of the World Medical Association (Declaration of Helsinki). The trial was registered with the European Clinical Trial Registry (Eudract 2014-000586-45) and the Belgian Federal Agency for Medicines and Health Products.

A diagnosis of ASD was made by a multidisciplinary team (child psychiatrist and/or expert neuropediatrician, psychologist, and speech/language pathologist and/or physiotherapist) based on strict DSM-IV-TR criteria. Before the study, the Autism Diagnostic Observation Schedule (27) and estimates of intelligence (6-subtest short version of the Wechsler Adult Intelligence Scale-IV, Dutch version) were acquired from all participants (Table 1). Detailed information on sample size and eligibility criteria is provided in the Supplemental Methods and Supplemental Table S1.

All assessments took place in the University Hospital of Leuven. Before the MRI scanning session, participants completed self-report questionnaires assessing social responsiveness (Social Responsiveness Scale [SRS]) (28,29) and repetitive behaviors (Repetitive Behavior Scale–revised [RBS-R]) (30). The State Adult Attachment Measure (SAAM) (31) and the Inventory of Parent and Peer Attachment (IPPA) (32) were also acquired to assess interindividual variations in state and trait attachment, respectively (see Supplemental Methods for a detailed description of the adopted questionnaires).

Nasal Spray Administration

Participants were randomly assigned to receive a single dose of OT or placebo based on a computer-generated randomized order. Except for the manager of randomization and masking of drug administration, all patients and research staff conducting the trial were blinded to treatment allocation. OT (Syntocinon; Sigma-Tau Industrie Farmaceutiche Riunite, Rome, Italy) and placebo (saline natriumchloride solution) were administered in amber 15-mL glass bottles with metered pumps (ACA Pharma, Nazareth, Belgium). A fixed dose of 24 IU was adopted, which is in accordance with most studies of intranasal OT in adults (3). Each puff per nostril contained 4 IU of OT. Participants received clear instructions to self-administer the nasal spray (3 puffs/nostril; 24 IU of OT) (33,34).

Assessment of Endogenous Salivary Oxytocin

Saliva samples were collected using the absorbent device technique (cotton swabs) right before and approximately 60 to

Table 1. Demographic and Clinical Characteristics of Participants Randomized to Receive Oxytocin or Placebo

	Oxytocin (n = 21)	Placebo (n = 17)	t	p Value
Age, Years, Mean ± SD	24.76 ± 4.85	24.06 ± 5.54	0.42	.68
Handedness, Right/Left, n	16/5	15/2		
IQ, Mean ± SD				
Total IQ	101.76 ± 12.52	107.29 ± 18.91	−1.08	.29
Verbal IQ	105.57 ± 9.27	111.35 ± 12.99	−1.60	.12
Performance IQ	104.76 ± 18.35	104.41 ± 21.88	0.05	.96
ADOS Score, Mean ± SD				
Total	7.19 ± 4.312	7.59 ± 3.89	−0.29	.77
Communication	2.14 ± 1.35	2.24 ± 1.44	−0.20	.84
Social interaction	5.05 ± 3.41	5.35 ± 3.14	−0.28	.78
RRB	1.19 ± 1.29	1.06 ± 0.9	0.36	.72
			Pearson χ^2	p Value
Use of Psychostimulant Medication[a], n	5	2	0.91	.34
Comorbidity[a], n	7	2	2.42	.12

ADOS, Autism Diagnostic Observation Schedule; RRB, restricted and repetitive behavior.
[a]Detailed information on medication use and comorbidities is provided in Supplemental Table S1.

70 minutes after nasal spray administration (a single dose of OT or placebo). Before collection, subjects were subject to oral resting (no eating, chewing gum, smoking, or drinking) for approximately 45 minutes. Salivary OT levels were determined using a commercial enzyme immunoassay Oxytocin ELISA kit (Enzo Life Sciences, Inc, Farmingdale, NY) (for more detailed information, see Supplemental Methods and Supplemental Figure S2).

MRI Data Acquisition and Analysis

MRI scanning was performed before and after nasal spray administration. In healthy participants, the impact of a single dose of intranasal OT on social cognition is commonly evaluated using a 30- to 45-minute wait time before the experimental task (3,35). Accordingly, post-MRI scanning was initiated approximately 30 minutes after nasal spray administration.

A 3T magnetic resonance scanner (Philips Healthcare, Best, the Netherlands) was used to acquire 1) anatomical images and 2) 7-minute resting-state fMRI scans during which participants were instructed to relax (but not sleep), to keep their eyes open while staring at a white cross, and to think of nothing in particular. More detailed information on the scanning parameters, MRI data preprocessing, and head motion analyses is provided in the Supplemental Methods and Supplemental Figure S3.

A recent structural neuroimaging study explored the relationship between endogenous OT levels and brain morphology of the hypothalamus (where OT is produced) and other brain regions of the central oxytocinergic system (amygdala, hippocampus, nucleus caudatus, and nucleus accumbens) (25). In accordance with this previous study, we performed between-subject regression analyses to explore the relationship between endogenous OT levels and region-to-region functional connectivity between a priori defined regions of interest (ROIs) centered over the hypothalamus, bilateral amygdala, hippocampus, nucleus caudatus, and nucleus accumbens (Supplemental Figure S4). All ROIs (n = 9) were defined from the subcortical FSL Harvard-Oxford atlas, except for the hypothalamus, which was defined with a sphere centered on Montreal Neurological Institute coordinates (0, −4, −8) using a 12-mm radius (36). For each participant, mean time series were extracted by averaging across all voxels in each ROI region and bivariate correlation coefficients were computed between each pair of ROIs. The resultant correlation values were Fisher z-transformed. As implemented in the CONN toolbox, a seed-level correction (false discovery rate [FDR]) was applied to correct for multiple comparisons. In addition to the hypothesis-driven analysis, we also performed a whole-brain regression analysis to identify associations between endogenous OT levels and region-to-region functional connectivity between all regions of the cortical (n = 91) and subcortical (n = 15) Harvard-Oxford atlas (FDR-corrected for multiple comparisons).

Statistical Analysis

Between-subject regression analyses were performed to identify relationships between endogenous OT levels and behavioral/neural (connectivity) measures. Since negative associations were identified between endogenous OT levels and amygdala–hippocampal connectivity (at baseline), we specifically explored whether a single-dose administration of OT would further reduce amygdala–hippocampus connectivity. To do so, pre-to-post–difference scores were calculated for each amygdala–hippocampal connection, and difference scores were subjected to a linear mixed-effect model with the random factor "subject" (n = 38) and the fixed factors "treatment" (OT and placebo), "amygdala ROI" (left and right), and "hippocampus ROI" (left and right).

RESULTS

Relationship Between Salivary OT and Behavior

Between-subject regression analyses revealed a significant relationship between endogenous salivary OT levels and self-reported attachment toward peers (IPPA subscale Peers; β = .62; t_{32} = 4.46; $p_{\text{uncorrected}}$ = .0001; $p_{\text{Bonferroni}} < .001$), indicating that participants with higher endogenous OT levels experience

more secure attachment toward peers (Figure 1A). No significant associations were identified for the Mother ($\beta = .05$) and Father ($\beta = -.09$) IPPA subscales.

For the SAAM, a similar positive relationship was revealed between feelings of secure attachment (SAAM Security subscale) and endogenous OT levels ($\beta = .52$; $t_{32} = 3.40$; $p_{uncorrected} = .005$; $p_{Bonferroni} < .05$) (Figure 1A). For the SAAM Avoidance subscale, a tentative negative relationship was revealed ($\beta = -.30$; $t_{32} = -1.77$; $p_{uncorrected} = .09$), indicating that participants with lower OT levels reported more feelings of attachment avoidance (Figure 1A). No significant association was identified for the Anxiety subscale ($\beta = .14$; $p > .05$). In addition, no significant associations were identified between endogenous levels of OT and the behavioral scales assessing autism symptoms (Autism Diagnostic Observation Schedule: $\beta = -.04$; SRS: $\beta = -.16$; RBS: $\beta = .06$; all $p > .05$).

Exploration of relationships between the different behavioral scales indicated that higher SRS total scores (more impairment in social responsiveness) were significantly associated with reduced feelings of secure attachment (IPPA) toward peers ($r = -.47$; $p_{uncorrected} = .004$), mother ($r = -.55$; $p_{uncorrected} = .001$), and father ($r = -.39$; $p_{uncorrected} = .025$) but not with reports of state attachment (SAAM) (security: $r = -.16$; avoidance: $r = .24$; anxiety: $r = .04$; all $p_{uncorrected} > .05$). Notably, exploratory analyses also showed that as a group, the patients with ASD had higher SRS scores ($t_{72} = -5.99$; $p_{uncorrected} < .001$), higher SAAM attachment avoidance ($t_{72} = -2.95$; $p_{uncorrected} = .021$), reduced SAAM attachment security ($t_{72} = 2.44$; $p_{uncorrected} = .017$), and a trend toward reduced feelings of secure attachment toward peers (IPPA) ($t_{72} = 1.63$; $p_{uncorrected} = .10$) when compared with behavioral characterizations previously obtained from neurotypical individuals

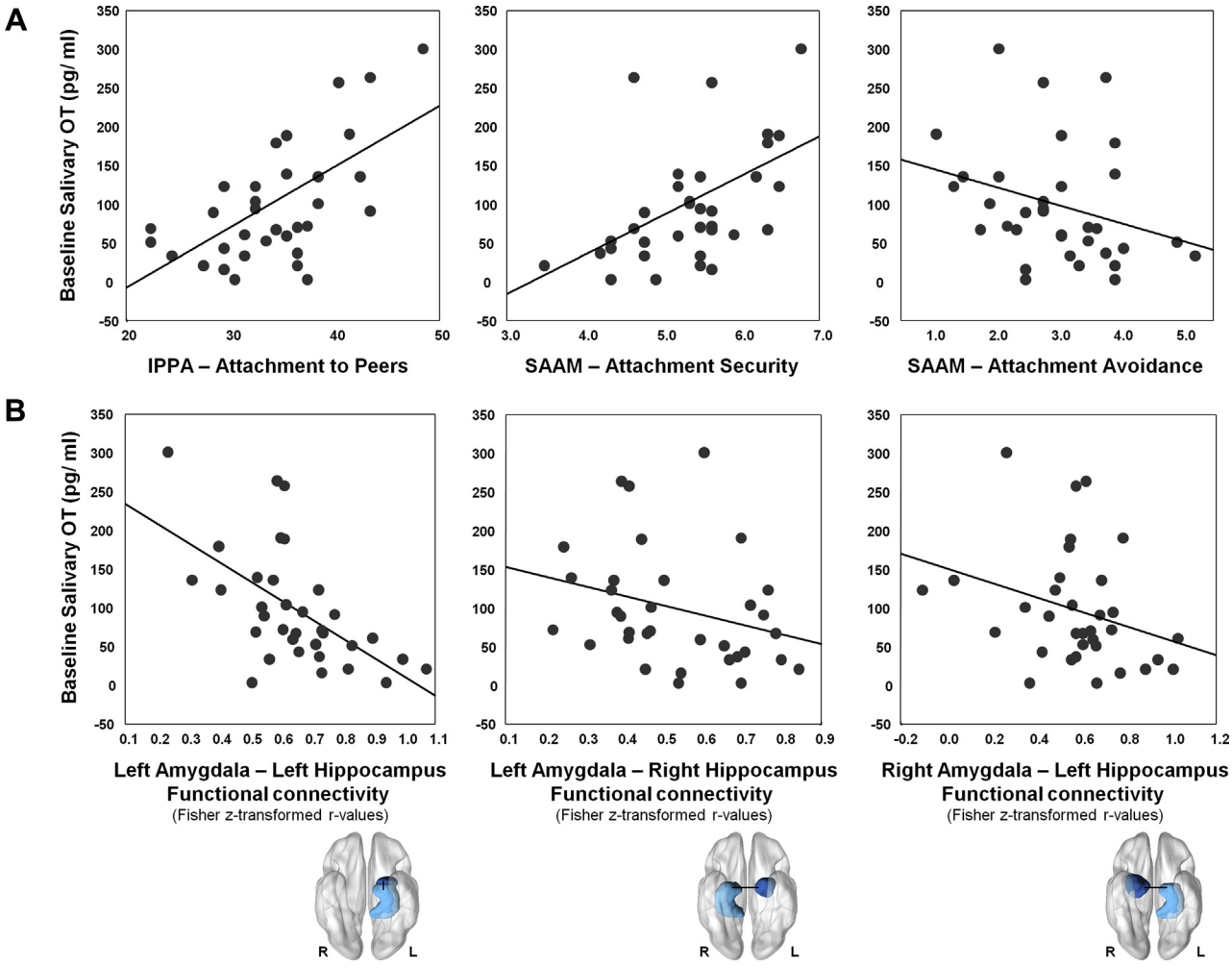

Figure 1. Association of endogenous oxytocin (OT) levels with behavior and resting-state functional connectivity. **(A)** Higher levels of endogenous OT were associated with expressions of secure attachment assessed with the Inventory of Parent and Peer Attachment (IPPA) Peers subscale and the State Adult Attachment Measure (SAAM) Security subscale. An inverse relationship was shown between endogenous OT levels and the SAAM Attachment Avoidance subscale. **(B)** At the neural level, higher levels of endogenous OT were associated with lower degrees of interregional functional connectivity between the amygdala and hippocampal regions. L, left; R, right.

(mean age ± SD, 21.1 ± 2.6 years) [data adopted from Bernaerts *et al.* (34)].

Relationship Between Salivary OT and Functional Connectivity

Between-subject regression analysis identified a significant relationship between endogenous OT levels and functional connectivity between the left amygdala and the left hippocampus ($\beta = -.60$; $t_{32} = -4.13$; $p = .0001$; $p_{FDR} = .001$), indicating that participants with higher OT levels display reduced amygdala–hippocampal connectivity (Figure 1B). At a $p_{uncorrected} < .05$ threshold, similar associations were identified between endogenous OT levels and connectivity between the left amygdala and the right hippocampus ($\beta = -.29$; $t_{32} = -1.71$; $p_{uncorrected} = .048$) and connectivity between the right amygdala and the left hippocampus ($\beta = -.30$; $t_{32} = -1.75$; $p_{uncorrected} = .044$) (Figure 1B). No significant associations were revealed for connectivity with or between the other ROIs (the nucleus caudatus, nucleus accumbens, and hypothalamus; all $p_{uncorrected} > .05$). Note that similar associations with amygdala-hippocampal connectivity were identified when regressions were performed with age and total IQ as covariates.

Whole-brain regression analysis consistently identified the negative association between endogenous OT levels and the left amygdala–left hippocampus connection and also identified a similar negative association for connectivity between the left amygdala and the left anterior parahippocampal gyrus ($\beta = -.55$; $t_{32} = -3.68$; $p_{uncorrected} = .0004$; $p_{FDR} = .044$).

Considering the identified association between OT levels and amygdala–hippocampal connectivity ($\beta = -.60$), as well as the aforementioned association between OT levels and interindividual variance in attachment security (IPPA [$\beta = .62$]; SAAM [$\beta = .52$]), we specifically explored whether variance in amygdala–hippocampal connectivity would also be associated with interindividual variation attachment security. Regression analyses identified a negative relationship between the extent of left amygdala–left hippocampus connectivity and attachment security (IPPA peers: $\beta = -.45$; $t_{32} = -2.83$; $p = .008$; SAAM: $\beta = -.42$; $t_{32} = -2.61$; $p = .01$), indicating that participants with higher attachment security also show lower levels of amygdala–hippocampal connectivity.

Effect of Exogenous OT Administration on Amygdala–Hippocampal Connectivity

As expected, after nasal spray administration, salivary OT concentrations were significantly augmented in the OT group but not in the placebo group ($F_{1,28} = 35.58$; $p < .0001$) (Figure 2A).

Linear mixed-effect analyses, exploring whether a single dose of OT could further reduce amygdala–hippocampal connectivity, revealed a tentative effect of treatment (across connections) ($F_{1,36} = 2.00$; $p = .08$ [one-sided]; $\eta_p^2 = .05$), as well as a trend level treatment × amygdala ROI interaction ($F_{1,36} = 2.14$; $p = .07$ [one-sided]), indicating that a single dose of OT significantly reduced connectivity of the right amygdala to the hippocampal regions ($p = .008$, Bonferroni post hoc test), but not connectivity of the left amygdala to the hippocampal regions ($p > .05$) (Figure 2B). Note that at a whole-brain level, we revealed no differential changes in functional connectivity from pre-to-post–nasal spray administration in the OT versus the placebo group (none of the effects survived correction for multiple comparisons, all $p_{FDR} > .05$).

DISCUSSION

The current study revealed that in adult men with ASD, higher levels of salivary OT are associated with higher expressions of secure attachment but lower degrees of interregional functional coupling between the amygdala and hippocampal regions. Notably, administration of a single dose of OT was able to induce a further reduction in the degree of functional connectivity between these core components of the central oxytocinergic system.

Previous fMRI studies have examined the effects of acute stressors on changes in functional connectivity and identified transient increases in amygdala–hippocampal coupling to be associated with dampened cortisol responses both in response to immediate stress (37) and in the prolonged aftermath of stress (38). Considering that cortisol has been implicated in enhancing hippocampus-mediated inhibition of the hypothalamic-pituitary-adrenal axis (39), these prior findings of Kiem *et al.* (37) and Vaisvaser *et al.* (38) provided evidence that stronger amygdala–hippocampal coupling is associated with a decreased efficiency of the hypothalamic-pituitary-adrenal axis to re-establish homeostasis after (stress-induced) perturbation. Interestingly, Fan *et al.* (40) demonstrated that state-dependent increases in amygdala–hippocampal connectivity upon stressors were more pronounced in participants with higher reports of early-life maltreatment and, notably, that this positive association was moderated after the administration of intranasal OT.

In the current study, we extend these findings by showing that intrinsic (trait-like) levels of functional coupling between the amygdala and the hippocampus were higher in individuals with lower endogenous levels of salivary OT, and that both variables were associated with variations in secure attachment (i.e., more insecure attachment in individuals with higher connectivity/lower OT levels). With respect to the association with OT levels, it is currently unclear whether the observed variations in amygdala–hippocampal interactions were instrumental to the observed interindividual differences in OT levels (e.g., inhibition/facilitation of central release of OT by enhanced/reduced amygdala–hippocampal connectivity), or inversely, whether a higher availability of OT per se was instrumental to the observed variations in amygdala–hippocampal interactions. In favor of the first interpretation, several anatomical and electrophysiological studies provided indications that the anterior hippocampus can exert an influence on peripheral OT secretion (41,42)—although note that the exact influence of hippocampal pathways on central OT release is unclear. Conversely, and in line with the latter interpretation, we here showed that a single dose of exogenously administered OT was able to induce a reduction in amygdala–hippocampal coupling (particularly for connectivity of the right amygdala to hippocampal regions). While only peripheral OT levels were assessed, previous studies in humans (43), nonhuman primates (44–46), and rodents (47) consistently demonstrated elevated OT levels in the central nervous system after

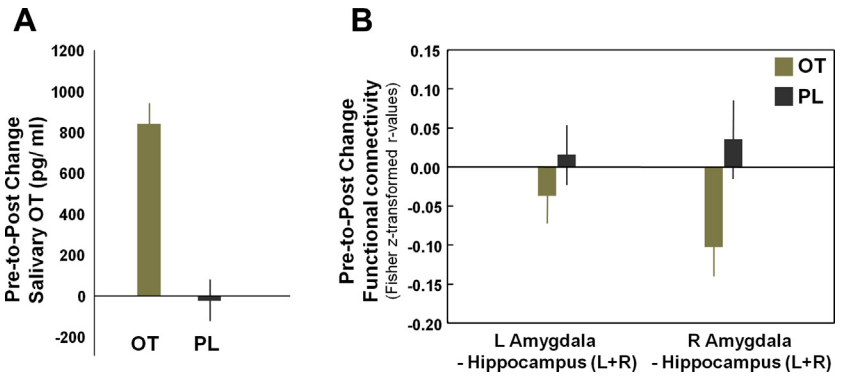

Figure 2. Effect of exogenous oxytocin (OT) administration on amygdala–hippocampal connectivity. **(A)** Change in salivary OT after nasal spray administration. **(B)** The effect of nasal spray administration on functional connectivity of the left (L) and right (R) amygdala to hippocampal regions is visualized separately for the OT and placebo (PL) groups.

exogenous administration of OT (48). These and our observations therefore indicate that an elevation of OT levels owing to nasal administration was instrumental and causally related to the observed changes in the degree of amygdala–hippocampal connectivity. While the exact pathways in the human brain are unknown, animal research identified axonal fibers containing OT in both the amygdala and hippocampal regions (49), suggesting a fast and focal pathway of OT-dependent neuromodulatory regulation in these regions. A recent meta-analysis of human neuroimaging studies identified OT-induced modulations in task-based brain activity of the amygdala and the parahippocampal gyrus as well as the superior temporal sulcus and caudate [see (50) for a recent review]. To date however, only a limited number of studies explored the effects of intranasal OT on intrinsic resting-state fMRI functional connectivity, and while a majority of studies reported enhanced amygdala resting-state connectivity (predominantly with anterior cingulate cortex and prefrontal cortex) (51–56), a number of studies reported decreased amygdala connectivity (52,54–57) or no effect (53,58,59) [see (60) for a recent review).

In terms of behavioral manifestations, higher endogenous OT levels and lower amygdala–hippocampal connectivity were shown to be associated with increased feelings of secure attachment (IPPA and SAAM). These observations are largely consistent with the implicated role of the OT system in parental bonding behaviors and attachment-related thoughts (22–24) and are also in line with the notion that early-life stress may impact on the oxytocinergic brain system. Indeed, research in rodents showed that the endogenous secretion of OT and the expression of OT receptors in the amygdala are modulated by the amount and quality of maternal care (61).

No significant associations were revealed between endogenous OT levels and the degree of core autism symptoms (social responsiveness or repetitive behaviors), which is in line with a previous report demonstrating no associations between baseline OT levels and expressions of autistic traits in the typical population (35). While to some extent impairments in social responsiveness were shown to be associated with reduced feelings of parent and peer attachment (IPPA), our data indicate that as a construct, interindividual variance in attachment style (state and trait attachment security) may be more sensitive for predicting aberrant oxytocinergic signaling as opposed to core autism symptoms. In the present study, salivary OT levels were only assessed for ASD patients (not for a control group), and it was therefore not possible to establish whether, on average, OT levels were lower in the current ASD patient sample compared with a sample of neurotypical individuals, as demonstrated before in previous studies (15–17) [although several exceptions should be noted (18–20)]. Exploratory analyses showed, however, that in terms of behavioral characterizations, patients with ASD significantly differentiated from neurotypical individuals on the assessments of attachment style (SAAM attachment security, SAAM attachment avoidance, and a trend for IPPA trait attachment toward peers), as well as (and as to be expected) in terms of social responsiveness. Previous trials investigating the efficacy of long-term (multiple-dose) OT treatment in patients with ASD mostly included evaluations of core autism symptoms (e.g., SRS, RBS, the Autism Diagnostic Observation Schedule), with some studies reporting treatment-induced improvements (9–11,14) and others failing to show beneficial effects (12,13). In view of the current identification of a tight association between attachment-related constructs and the oxytocinergic system in patients with ASD, as well as results from a previous study from our laboratory reporting significant improvements in attachment avoidance and peer attachment in neurotypicals after a 2-week course of continual OT treatment (34), we anticipate that it would be of high relevance for future OT trials with ASD patients to continue to include characterizations of attachment style for evaluating treatment outcome. Indeed, since interindividual variance in attachment style has been highlighted as a potential modulator of OT treatment effects (2,62), it is anticipated that a thorough characterization of attachment style in patients with ASD will aid in explaining interindividual differences in treatment responses and/or delineating patient populations that may benefit the most from a course of OT treatment.

Although our study provides new insights regarding the association of endogenous OT levels with behavioral manifestations and intrinsic functional couplings between key nodes of the central OT system, several limitations need to be considered. First, while core autism symptoms were assessed based on observational scales (Autism Diagnostic Observation Schedule) and self-report questionnaires (SRS and RBS), variations in attachment were solely assessed based on self-report questionnaires (SAAM and IPPA). Further, some variation existed in the time point of collection of the salivary

samples for assessing OT concentration, and a tentative negative relationship was revealed indicating slightly higher concentrations for the participants tested in the morning compared with participants tested in the afternoon/evening (Supplemental Figure S2). Note, however, that all the identified associations remained significant after correction for the timing of salivary collection, indicating that the reported relationships persisted over and above variations in OT concentration related to the time point of collection. Further, while a recent study demonstrated moderate to strong correlations between salivary and central (cerebrospinal fluid) OT levels (63), it should be noted that it remains currently unclear how peripheral OT measures are functionally related to central OT activity. Finally, and as stated before, we also note the lack of a control group of neurotypical individuals as well as the relatively small sample size of included patients with ASD as a limitation of the current study. As such, future research may be warranted to further explore whether similar associations between endogenous OT levels and amygdala-hippocampal circuits are evident in neurotypical or other neuropsychiatric populations.

In summary, we found that higher levels of endogenous OT are associated with lower levels of functional connectivity between two core nodes of the central oxytocinergic system (the amygdala and hippocampal regions) and that both variables were associated with expressions of secure attachment, but not core autism symptoms, in male adults with ASD. Notably, the observation that a single dose of OT was able to induce a reduction in amygdala–hippocampal connectivity indicates that a higher availability of OT causally affected the degree of amygdala–hippocampal connectivity. Together, these findings are in line with the growing body of research highlighting the amygdala and hippocampal regions (and their intrinsic interaction) as key neural loci of the central OT system. In line with previous findings evidencing a link between amygdala–hippocampal connectivity and central responses to stress (i.e., capacity of the hypothalamic-pituitary-adrenal axis to restore homeostasis after perturbation) (39,40,42), the identified associations between the oxytocinergic system and amygdala–hippocampal pathways are anticipated to be of relevance for understanding the role of OT in modulating appropriate neural and physiological responses to stress and restoring homeostasis. An increased vulnerability to cumulative stress can hugely impact well-being and induce deteriorations in several domains (e.g., social functioning), and therefore future research is urged to further explore, both from a neural perspective and from a behavioral perspective, the role of the endogenous oxytocinergic system in adequate stress responses and maintenance of homeostasis and to characterize the effect of exogenously administered OT on these processes.

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ClinicalTrialsRegister.eu: Oxytocin-Based Pharmacotherapy for Autism Spectrum Disorders: Investigating the Neural and Behavioral Effects of a Promising Intervention Approach; https://www.clinicaltrialsregister.eu/ctr-search/search?query=2014-000586-45; 2014-000586-45.

ARTICLE INFORMATION

From the Department of Rehabilitation Sciences (KA, SB, ND), Group Biomedical Sciences, Neuromotor Rehabilitation Research Group, and the Department of Chronic Diseases (BV), Metabolism and Ageing, Group Biomedical Sciences, Pneumology Research Group, University of Leuven, Katholieke Universiteit Leuven, Belgium, and the Department of Health Sciences and Technology (NW), Neural Control of Movement Lab, Eidgenössische Technische Hochschule, Zurich, Switzerland.

Address correspondence to Kaat Alaerts, Ph.D., Neurorehabilitation Research Group, Department of Rehabilitation Sciences, Group Biomedical Sciences, University of Leuven, Katholieke Universiteit Leuven, Belgium, Tervuursevest 101, B-3001 Heverlee, Belgium; E-mail: Kaat.Alaerts@kuleuven.be.

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